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(54) Title: SUBSTRATES FOR O⁶-ALKYLGUANINE-DNA ALKYLTRANSFERASE

(57) Abstract: The present invention relates to methods of transferring a label from novel substrates to O⁶-alkylguanine-DNA alkyltransferases (AGT) and O⁶-alkylguanine-DNA alkyltransferase fusion proteins, and to novel substrates suitable in such methods. Proteins of interest are incorporated into an AGT fusion protein, the AGT fusion protein is contacted with particular AGT substrates carrying a label, and the AGT fusion protein is detected and optionally further manipulated using the label in a system designed for recognising and/or handling the label. The particular AGT substrates used in the method of the invention are O⁶-substituted guanine derivatives or related nitrogen containing hydroxy-heterocycles and their sulfur analogs wherein the O⁶-substituent is an activated methyl derivative suitable for transfer from guanine or the corresponding heterocycle to AGT, and further carrying a label or a plurality of same or different labels. The invention relates also to the novel AGT substrates as such, to methods of manufacture of such novel substrates, and to intermediates useful in the synthesis of such novel AGT substrates.

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